IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	**09/707,117**	Confirmation No. 8513
Applicants	:	**Jon A. Wolff, Vladimir Budker**	
Filed	:	**11/06/2000**	
Art Unit	:	**1632**	
Examiner	:	**Wilson, Michael C.**	
Docket No.	:	**Mirus.018.01**	

For: **Intravascular Delivery of Nucleic Acid**

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Dr. Zane Neal, hereby declare as follows:

1. I have a Doctorate in Cellular/Molecular Immunology from the University of Wisconsin, Madison.
2. I am familiar with the above captioned application and the Budker et al. (1998, Gene Therapy, Vol. 5, pg 272-276) publication.
3. I am the author of the attached statement regarding the effect of collagenase on the immune system.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-27-05

Dr. Zane Neal Date

Re: Collagenase functioning as an immunosuppressive agent

In the patent application 09/707,117, the Action suggests that inclusion of collagenase in the invention for delivery of nucleic acids to skeletal muscle, may be considered to serve as an immunosuppressive agent. The Examiner is correct in interpreting that collagenase action affects the blood vessels by promoting increased permeability, transiently mimicking conditions similar to vascular leak syndrome or capillary leak syndrome. The Examiner's reasoning that diminished blood flow could impact immune function by decreasing trafficking of immune effector cells through the lymphatic system is unprecedented. A cursory review of the literature using Medline and PubMed failed to identify any scientific studies reporting such a phenomenon.

In my research experience understanding the immune response to infection and preclinical tumor immunobiology, I am unaware of employing increased vascular permeability as a method to induce locoregional or systemic immunosuppression. If loss in blood flow through the isolated limb during the delivery procedure was severe enough to negatively impact the immune status of the host by affecting the flow rate through the lymphatics, we would expect to observe signs of acute ischemia in the isolated limb.

Conversely, vascular damage and increased vascular permeability are more likely to induce immune activation by initiating immune inflammatory responses. The response to trauma begins in the immune system at the moment of injury. The loci are the wound, with activation of macrophages and production of proinflammatory mediators, and the microcirculation with activation of endothelial cells (EC), blood elements, and a capillary leak. These processes are potentiated by areas of micro-ischemia and impaired oxygen delivery and by the presence of necrotic tissue, each exacerbating the inflammatory response (Seminars in Pediatric Surgery 4:77-82, 1995). ECs are able to produce vasodilatory mediators and several factors lead to increased vascular permeability (i.e., similar to the effects of collagenase). ECs play a central role in immune cell extravasation, a key feature of inflammation (Rheumatic Diseases Clinics of North America 30:97-114, 2004). Unlike the patent reviewers position, I would anticipate use of collagenase to increase vascular permeability should promote an immediate inflammatory response and stimulation of immune system components.

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